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Short communication

R-citalopram attenuates anxiolytic effects of escitalopram in a rat ultrasonic vocalisation model

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Abstract

Escitalopram mediates the serotonin reuptake inhibitory effect of citalopram. To investigate the potential interactive effects between escitalopram and *R*-citalopram, they were studied at standard and elevated serotonin levels in a model predictive of anxiolytic activity (inhibition of footshock-induced ultrasonic vocalisation in adult rats). At standard levels, citalopram partially inhibited (64%) and escitalopram abolished (97%) vocalisation. Co-treatment with L-5-hydroxytryptophan resulted in complete inhibition with citalopram and a substantially enhanced response to escitalopram, while *R*-citalopram increased the vocalisation significantly. Furthermore, *R*-citalopram attenuated the effect of escitalopram. These findings may be relevant to the enhanced clinical efficacy seen with escitalopram compared to citalopram.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Anxiolytic; Citalopram; Escitalopram; *R*-citalopram

1. Introduction

The selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors have gained extensive clinical use during the last two decades and are drugs of choice for the treatment of depressive and anxiety disorders. The widely used selective 5-HT reuptake inhibitor, citalopram, is a racemic mixture of *S*-(+)- and *R*-(−)-enantiomers (escitalopram and *R*-citalopram, respectively) in a 1:1 ratio. The 5-HT reuptake inhibitory activity of citalopram has previously been reported to reside in the *S*-enantiomer (Hyttel et al., 1992). During the last few years, escitalopram has been successfully used to treat major depression and anxiety disorders (Montgomery et al., 2001; Burke et al., 2002; Wade et al., 2002).

The in vitro and in vivo 5-HT reuptake inhibitory activity and the effect of escitalopram in animal models of depression, anxiety and aggressive behaviour has recently been characterised and compared with citalopram (Sánchez et al., in press). In the above-mentioned study, escitalopram and citalopram provoked different responses using footshock-induced ultrasonic vocalisation in rats—a model that mimics aspects of panic anxiety (Sánchez, in press). While escitalopram was able to inhibit footshock-induced ultrasonic vocalisation completely, citalopram only produced a partial inhibition in the same dose range. *R*-citalopram also partially inhibited footshock-induced ultrasonic vocalisation but was several times less potent than escitalopram and citalopram. The partial inhibition produced by citalopram and the biphasic nature of its dose–response could not be explained readily. One possible mechanism could be that *R*-citalopram interferes with the action of escitalopram as the basal level of 5-HT increases. The present study was designed to investigate the inhibitory effect of citalopram and escitalopram on footshock-induced ultrasonic vocalisation in rats at normal and increased levels of 5-HT. The latter condition can be brought about by concomitant treatment with a low dose (25 mg/kg) of the 5-HT precursor, L-5-hydroxytryptophan (L-5-HTP). Furthermore, the effects of *R*-citalopram on escitalopram-induced inhibition of footshock-induced ultrasonic vocalisation were investigated.

2. Material and methods

2.1. Animals, housing conditions and ethics

Adult male Wistar WU rats (starting weight 150–175 g: Charles River, Germany) were housed in groups of two to

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Table 1
Inhibition of footshock-induced ultrasonic vocalisation in adult rats by citalopram and escitalopram, at normal and enhanced levels of 5-HT, achieved after administration of a non-inhibitory dose of L-5-HTP (25 mg/kg, s.c.)

Administration (min before shock regimen)	Drug (30 min), s.c.		Drug (50 min) + l-5HTP (25 mg/kg, 20 min), s.c.	
	ED_{50} (mg/kg)	Max inhibition (%)	ED_{50} (mg/kg)	Max inhibition (%)
Citalopram	ND[a]	64	1.1 0.69–1.8	100
Escitalopram	0.51 0.34–0.77	97	0.052 0.033–0.082	100

Four 1.0 mA inescapable footshocks, each of 10 s duration and with 5 s intershock intervals were applied. One min after the last shock the accumulated time spent emitting ultrasounds was measured during a 5 min period. Results are expressed as ED_{50} values with 95% confidence intervals and % maximum inhibition. ND = not determined, a: biphasic response with maximum inhibition at 0.50 mg/kg.

four in Macrolon cages type III. They were habituated to the animal facilities for at least a week before the start of the experiments. The room temperature (21 ± 2 °C), relative humidity (55 ± 5%), and air exchange (16 times/h) were automatically controlled. The animals had free access to commercial food pellets and tap water between test sessions. The study was conducted in compliance with the EC Directive 86/609/EEC and with Danish law regulating experiments on animals. Ethical permission was granted by the animal welfare committee, appointed by the Danish Ministry of Justice.

2.2. Footshock-induced ultrasonic vocalisation

The experiments were conducted as previously described in detail by Sánchez (1993). Briefly, Perspex test cages (22 × 22 × 22 cm) with a metal grid floor were used and footshocks were delivered from a two-pole shocker. A microphone, sensitive to ultrasound in the range of 20–30 kHz, was placed in the centre of the lid of the test cage. The ultrasounds were preamplified and converted from AC signals to DC signals using a signal rectifier. The accumulated time in which the voltage of the rectified signal was larger than the voltage of a previously determined threshold level was recorded.

Animals were primed with the shock regimen 24 h before the first test session. Immediately after being placed in the test cage, the rats received four 1.0-mA inescapable footshocks, each of 10-s duration and with an intershock interval of 5 s. They were left in the test cages for 6 min after the last shock. The same shock regimen was followed on test days. Recording of ultrasonic vocalisation started 1 min after the last shock and lasted for 5 min. For each test session, the animal groups were randomly allocated to treatment with saline or test drug. Each treatment group consisted of eight animals. One saline group and two to four drug-treated groups were included at each session. Each

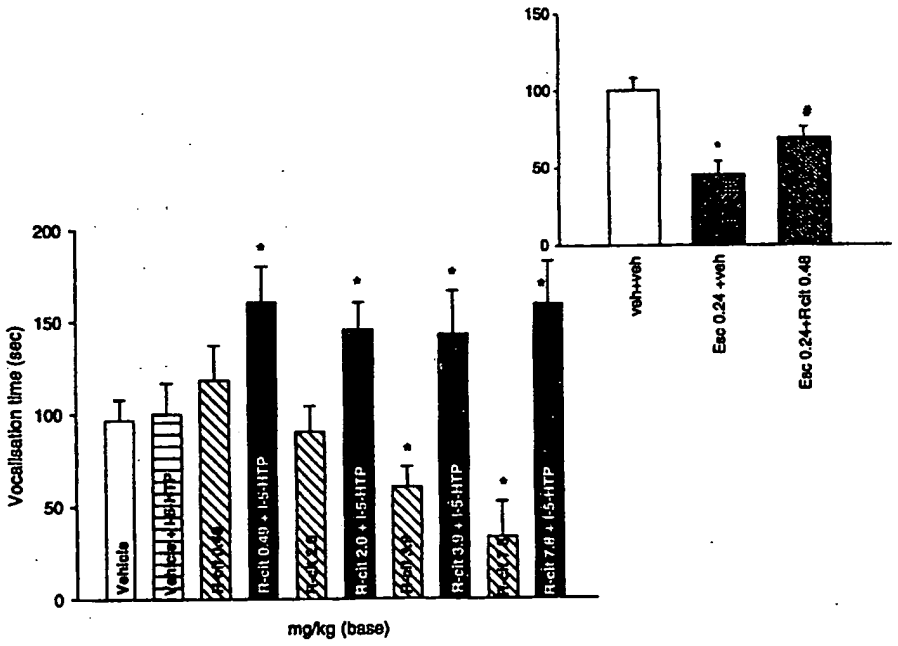

Fig. 1. Effect of *R*-citalopram on footshock-induced ultrasonic vocalisation in adult rats with enhanced levels of 5-HT. *R*-citalopram and L-5-HTP, 25 mg/kg, was administered s.c. 50 and 20 min, respectively, before test. Four 1.0-mA inescapable footshocks, each of 10-s duration and with 5-s intershock intervals were applied. One min after the last shock, the accumulated time spent emitting ultrasounds was measured for 5 min. Results are shown as mean ± S.E.M. $^{*}P<0.05$ compared to control. (Insert) Effect of *R*-citalopram on escitalopram-induced inhibition of footshock-induced ultrasonic vocalisation in adult rats. *R*-citalopram and escitalopram were administered s.c. as separate injections 30 min before test. Results are shown as mean ± S.E.M. $^{\#}P<0.05$ compared to escitalopram alone and $^{*}P<0.05$ compared to vehicle.

drug was tested in at least two separate experiments with overlapping doses. The rats were tested on a weekly basis for up to 7–8 weeks.

2.3. Drug treatment

Citalopram HBr, escitalopram oxalate and *R*-citalopram oxalate (all synthesised in the Department of Medicinal Chemistry, H. Lundbeck) and L-5-hydroxytryptophan (L-5-HTP) (Sigma, USA) were dissolved in saline. The injection volume was 5 ml/kg body weight. Injections were administered s.c. All doses were expressed in mg base/kg body weight.

2.4. Statistics

Results were analysed by one-way analysis of variance followed by post hoc comparison of means (Tukey's test). *P*-values less than 0.05 were considered statistically significant. The software package SigmaStat™ for Windows™ (Jandel, San Rafael, CA, USA) was used. ED_{50} values with 95% confidence limits were calculated using log-probit analyses.

3. Results

Under standard test conditions, citalopram only partially inhibited ultrasonic vocalisation, while escitalopram abolished the response (Table 1). When rats in addition were treated with L-5-HTP (25 mg/kg) to increase basal levels of 5-HT, the response to citalopram was slightly attenuated, while the response to escitalopram was markedly enhanced, the ED_{50} value being approximately 10-fold lower than the standard test conditions (Table 1). *R*-citalopram inhibited ultrasonic vocalisation with much lower potency than escitalopram (ED_{50} = 6.8 mg/kg) and concomitant treatment with *R*-citalopram and L-5-HTP (25 mg/kg) produced a significant increase of footshock-induced ultrasonic vocalisation compared to controls (Fig. 1).

The ability of *R*-citalopram to attenuate the anxiolytic-like effect mediated by escitalopram was tested further by comparing the footshock-induced ultrasonic vocalisation in rats dosed with escitalopram alone and those dosed with escitalopram (0.24 mg/kg) and *R*-citalopram (0.48 mg/kg) concomitantly. The addition of *R*-citalopram significantly attenuated the inhibitory effect of escitalopram on footshock-induced ultrasonic vocalisation (insert, Fig. 1).

4. Discussi n

The results of the present studies show that *R*-citalopram attenuates escitalopram-induced inhibition of ultrasonic vocalisation, and this attenuating effect is enhanced at raised levels of 5-HT. The effect of *R*-citalopram is observed at doses that are below those that inhibit ultrasonic vocalisation (Fig. 1). The mechanism by which *R*-citalopram modulates escitalopram-induced inhibition of ultrasonic vocalisation is, however, not known at the moment.

Various studies suggest that footshock-induced ultrasonic vocalisation in rats involves presynaptic 5-HT_{1A} receptors (Remy et al., 1996; Sánchez et al., 1996). For example, the 5-HT_{1A} receptor agonists, 8-hydroxy-2(di-*n*-propylamino)-tetralin (8-OH-DPAT) and buspirone, potently inhibit footshock-induced ultrasonic vocalisation. However, citalopram and its enantiomers do not have any significant affinity for 5-HT_{1A} receptors as shown in receptor binding studies performed with rat brain homogenate (Sánchez et al., in press). It is, therefore, highly unlikely that *R*-citalopram modulates presynaptic 5-HT_{1A} receptor activity by a direct effect on these receptors.

Of the 144 targets tested in in vitro binding affinity studies (Sánchez et al., in press), the only site for which citalopram and *R*-citalopram, but not escitalopram, showed appreciable affinity was that of the histamine H_1 receptor. In vitro studies in isolated guinea pig ileum show that *R*-citalopram is a weak histamine H_1 receptor antagonist (unpublished observation). Histamine H_1 receptors are involved in mediation of ultrasonic vocalisation and the histamine H_1 receptor antagonist, mepyramine, antagonises footshock-induced ultrasonic vocalisation (Sánchez, in press). Thus, it would be expected that *R*-citalopram's histamine H_1 receptor antagonistic activity would enhance rather than attenuate the effect of escitalopram on footshock-induced ultrasonic vocalisation.

The possibility that *R*-citalopram is modulating the effect of escitalopram by having an effect on a hitherto unidentified receptor cannot be excluded. Another possibility is that *R*-citalopram modulates the interaction of escitalopram with the 5-HT transporter protein and thereby affects 5-HT levels at the synapse. This is consistent with the observation that the effect of *R*-citalopram on ultrasonic vocalisation depends on the endogenous 5-HT level. *R*-citalopram is 30- to 100-fold less potent than escitalopram in its ability to inhibit 5-HT reuptake in vitro and *R*-citalopram is practically devoid of in vivo 5-HT uptake inhibitory activity measured as potentiation of 5-HTP-induced behavioural changes (Owens et al., 2001; Sánchez et al., in press). However, previously published studies of in vitro binding kinetics of [^{3}H]citalopram in rat brain homogenates and human platelets showed that higher concentrations of escitalopram or *R*-citalopram stabilises the binding of [^{3}H]citalopram, resulting in a low dissociation rate (Plenge and Mellerup, 1985). This is suggested to be due to an allosteric effect on the 5-HT transporter protein via binding to a low-affinity site and is a unique property of citalopram (Plenge et al., 1991). Recent studies in membrane preparations of monkey kidney cells (COS-1 cells) expressing human 5-HT transporter protein have compared effects of escitalopram and *R*-citalopram on dissociation rates of [^{3}H]escitalopram, [^{3}H]*NN*,dimethyl-2-(2-amino-4-methylphenylthio)benzyl-

amine ([^{3}H]MADAM) and [^{125}I]2β-carbomethoxy-3β(4-iodophenyl)tropane ([^{125}I]RTI) and have demonstrated differences between escitalopram and *R*-citalopram (Wiborg and Sánchez, 2002). The potency ratio between the enantiomers was much lower than for 5-HT uptake inhibition, suggesting that the *R*-enantiomer may have a significant allosteric effect. Furthermore, the potency ratio depended on the radioligand studied, indicating that the enantiomers interact differently with the 5-HT transporter protein. A p ssible interference of *R*-citalopram on the binding kinetics of escitalopram and the relevance of this phenomenon at pharmacologically relevant concentrations in vivo remains to be established.

In conclusion, the *R*-enantiomer of citalopram attenuates the anxiolytic-like effect of escitalopram in a rat ultrasonic vocalisation model. The mechanism involved is unknown but may be related to the improved clinical antidepressant activity seen with escitalopram in comparison with citalopram (Gorman et al., 2002).

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